

# Magnesium is More Efficient than Calcium in Alleviating Aluminum Rhizotoxicity in Soybean and its Ameliorative Effect is not Explained by the Gouy-Chapman-Stern Model

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The mechanistic basis for cation amelioration of Al rhizotoxicity in soybean was investigated through a series of studies comparing protective effects of Ca and Mg against Al inhibition of root elongation in a background 0.8 mM CaSO<sub>4</sub> solution (pH 4.3). A modified Gouy-Chapman-Stern model was used to evaluate the effect of cations on electrical potential and Al<sup>3+</sup> activity at root plasma membrane surfaces. Activities of Al<sup>3+</sup> up to 4.6 μM in the background solution inhibited soybean tap root elongation by more than 80%. There was little or no response in root elongation when Ca and Mg were added to background solutions in the absence of Al. When added to Al-toxic solutions in the micromolar concentration range, Mg was 100-fold more effective than Ca in alleviating Al toxicity, whereas both cations were equally effective when added in the millimolar concentration range. The protective effect of micromolar additions of Mg on root elongation was specific for Al and it failed to alleviate La rhizotoxicity. In contrast to wheat, Mg amelioration of Al toxicity to soybean root elongation at low Mg concentration could not be explained by changes in potential and Al<sup>3+</sup> activity at the root plasma membrane surfaces as predicted by a Gouy-Chapman-Stern model. These results suggest that Mg is not acting as an indifferent cation when present at low concentration and implies the involvement of a mechanism other than pure electrostatic effects at the root surface.

**Key words:** Aluminum toxicity — Calcium — Cation amelioration — Gouy-Chapman-Stern model — Magnesium — Soybean.

Abbreviations: Al, aluminum; Ca, calcium; La, lanthanum; Mg, magnesium; Mn, manganese;  $\Psi_m$ , root cell surface electrical potential;  $\{M^{n+}\}_m$ , cation activities at the plasma-membrane surface.

## Introduction

Under acidic conditions Al is toxic to plant roots and reduces their capacity to absorb water and nutrients. The

severity of Al rhizotoxicity depends on the presence of other cations in solution (Gonzalez-Erico et al. 1979, Adams 1984, Bruce et al. 1988, Foy 1992). Research on the role of cations in modifying Al toxicity has focused on Ca and Mg and has been reported for a variety of plant species including soybean (Alva et al. 1986, Brady et al. 1993, Noble and Sumner 1988, Sanzonowicz et al. 1998a, Sanzonowicz et al. 1998b, Ferrufino et al. 2000). Nonetheless, hypotheses to explain beneficial effects of Ca and Mg beyond the solution ionic strength effects have remained largely speculative (Kinraide and Parker 1987, Edmeades et al. 1991).

Mechanistic description of cation amelioration of Al toxicity by a Gouy-Chapman-Stern model (Kinraide et al. 1992, Kinraide 1994, Kinraide 1998) has gained recent recognition. The model demonstrates that addition of salts to the growth medium increases electrical potential and reduces Al<sup>3+</sup> activity at plasma membrane surfaces of root cells (Kinraide et al. 1992, Kinraide 1994, Kinraide 1998). Model predictions match the order of effectiveness of the cations ( $M^{n+}$ ) in alleviating Al inhibition of root growth of wheat genotypes and are dependent on the following sequence:  $H^+ > M^{3+} > M^{2+} > M^{1+}$  (Kinraide and Parker 1987, Kinraide et al. 1992, Kinraide et al. 1994, Kinraide 1994).

Application of the Gouy-Chapman-Stern model has been restricted to comparisons of the Al-sensitive wheat genotypes Scout 66 and Tyler. The objectives of the present study were to characterize the effects of Ca and Mg on the Al-induced root growth inhibition of soybean genotypes with differential Al tolerance and relate differences in cation amelioration to the root cell surface electrical potentials and root plasma membrane toxicant activities as computed with a Gouy-Chapman-Stern model.

## Material and Methods

### Growth conditions

Soybean seedlings of cultivar Young and Plant Introduction 416937 (PI), previously ranked as Al-sensitive and Al-tolerant, respectively (Bianchi-Hall et al. 1998, Silva et al. 2000), were obtained by placing seeds between sheets of germination papers soaked with 0.1 mM CaSO<sub>4</sub>. Rolls of paper with the seeds were placed vertically into plastic beakers in an incubator in the dark at 25–26°C for 3 d.

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Germinated soybean seedlings were selected for uniformity of tap root length and transferred to hydroponic chambers containing 12 liters of a background 800  $\mu\text{M}$   $\text{CaSO}_4$  solution. Solution pH was continuously monitored and maintained at 4.3 with additions of 0.1 M  $\text{H}_2\text{SO}_4$  throughout the experimental period. Solutions were circulated at a rate of 5 liter  $\text{min}^{-1}$  to ensure adequate aeration and treatment uniformity. Lighting was provided by a combination of metal halide and sodium vapor lamps at an average photosynthetic photon flux density of 550  $\mu\text{mol m}^{-2} \text{s}^{-1}$  at the top of the chambers in a 8/16 h (day/night) photoperiod. Solution temperature, which was not controlled, remained at  $26 \pm 2^\circ\text{C}$  throughout all experiments.

#### *Solution treatments and computation of root cell electrical potential and ion activities*

All treatments were established in a background 800  $\mu\text{M}$   $\text{CaSO}_4$  (pH 4.3) solution in order to provide Ca required for optimum root growth (Sanzonowicz et al. 1998a, Sanzonowicz et al. 1998b). Aluminum was added to the culture solutions from a 100 mM  $\text{AlCl}_3$  stock solution in dilute HCl. Lanthanum was added as  $\text{LaCl}_3$  from a stock solution of similar concentration. Base was never added to the treatment solutions and special care was taken to avoid Al polymerization/precipitation as previously suggested (Kinraide 1991). All the potential ameliorant cations ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$ ) were added as chloride salts. Since the addition of ameliorating cations can increase the ionic strength and reduce the activity of  $\text{Al}^{3+}$  or  $\text{La}^{3+}$  in solution, concentrations of  $\text{AlCl}_3$  and  $\text{LaCl}_3$  were adjusted in order to keep a constant  $\text{Al}^{3+}$  or  $\text{La}^{3+}$  activity in the external medium ( $\{\text{Al}^{3+}\}_e$  and  $\{\text{La}^{3+}\}_e$ , respectively), as predicted by the GEOCHEM-PC software (Parker et al. 1995).

Independent estimates of free ion concentration in solutions, as predicted by GEOCHEM-PC, were used as inputs to compute root cell surface electrical potential ( $\Psi_m$ ) and cation activities at the plasma-membrane surface ( $\{\text{M}^{n+}\}_m$ ) according to a modified Gouy-Chapman-Stern model (Kinraide et al. 1998). Computations of ion activities in the external medium were compared between the Gouy-Chapman-Stern program and GEOCHEM-PC and provided close agreement in all experiments.

#### *Root elongation measurements*

After a 16–18 h acclimation period in the background solution, tap root length was measured with a ruler and solution treatments were imposed. Root elongation was determined by measuring the tap root length 72 h after exposure to solution treatments. For experiments involving lateral roots, the average value of the two longest lateral roots was used. In most experiments root elongation rate is expressed as a percent of control treatments, that is root elongation in treatments with Al relative to treatments containing the same composition without Al. For time course experiments, seedlings were removed from the solution chambers during root length measurement. In each treatment, root measurements were performed on 6–12 plants and experiments were carried out independently twice.

## **Results**

#### *Cell surface electrical potential and ion activities*

Addition of different toxic or ameliorative cations to the 800  $\mu\text{M}$   $\text{CaSO}_4$  background solution incurred significant changes in the computed electrical potential and ion activities at membrane surfaces (Table 1). In the background solution the computed root cell membrane potential was  $-22 \text{ mV}$  in the absence of Al and increased to  $-5.6 \text{ mV}$  at the highest Al level.

According to the model, the activity of Ca at the root surface decreased with increasing levels of  $\text{Al}^{3+}$ . Additions of Ca to the solution increased the computed root cell potential, decreased the predicted  $\{\text{Al}^{3+}\}_m$  and increased  $\{\text{Ca}^{2+}\}_m$ . Virtually no change in membrane potential and  $\{\text{Al}^{3+}\}_m$  were observed in the treatments with small additions of Mg or Mn. Supplying La to solution increased  $\Psi_m$  and  $\{\text{La}^{3+}\}_m$ , but to a smaller extent than that observed upon adding equivalent activities of  $\text{Al}^{3+}$  to the solutions (Table 1).

#### *Tap root elongation response to Ca and Mg*

Supplementing the 800  $\mu\text{M}$   $\text{CaSO}_4$  background solution with up to 1.0 mM Ca in the absence of Al promoted tap root elongation by 10–20% for cultivar Young and the PI. Root elongation of both genotypes was inhibited when  $\text{Al}^{3+}$  activity was 2.9  $\mu\text{M}$  in the background solution and greater responses were observed to supplementary Ca additions (Fig. 1A). Adding more Ca to the solution increasingly alleviated the inhibitory effects of Al. Although 3.0 mM Ca promoted tap root elongation in both genotypes, the relative root elongation of the Al-sensitive cultivar Young was less than that of PI. Although 1.0 mM Ca improved tap root elongation of the PI almost to that of control levels, a similar ameliorative effect in cv Young required 3.0 mM Ca (Fig. 1A).

In the absence of Al, root elongation response to Mg was limited to a 15% increase with 10  $\mu\text{M}$  Mg. Tap root elongation was inhibited by more than 70% in both genotypes when 2.9  $\mu\text{M}$   $\text{Al}^{3+}$  was present in the background solution (Fig. 1B). Adding 10  $\mu\text{M}$  Mg increased root growth in both genotypes with greater effects in the PI. Al toxicity was completely alleviated in both genotypes when 25  $\mu\text{M}$  Mg was present (Fig. 1B) and the response to supplementary Mg was equivalent to at least 3 mM Ca (compare Fig. 1A with Fig. 1B).

#### *Lateral roots response to Ca and Mg*

Based on previous reports that Al inhibits elongation of soybean lateral roots more than tap roots (Brady et al. 1993, Sanzonowicz et al. 1998a, Sanzonowicz et al. 1998b, Ferrufino et al. 2000, Silva et al. 2001a), we sought to determine if concentrations of Ca and Mg in solution required to alleviate Al toxicity differed among root classes. Although the solution  $\text{Al}^{3+}$  activities and Ca and Mg concentration ranges were similar to that for experiments evaluating tap roots, a distinct response pattern was observed for the laterals. Amelioration of Al inhibition of lateral root elongation was only evident when 3.0 mM Ca was added to the background solution, but root elongation of both genotypes was 60% less than for control treatments without Al (Fig. 2). In contrast to tap roots (Fig. 1B), the addition of 25  $\mu\text{M}$  Mg did not overcome Al inhibition to lateral root elongation; complete alleviation of Al toxicity was observed only at 50  $\mu\text{M}$  Mg.

#### *Magnesium amelioration of $\text{Al}^{3+}$ and $\text{La}^{3+}$ rhizotoxicity*

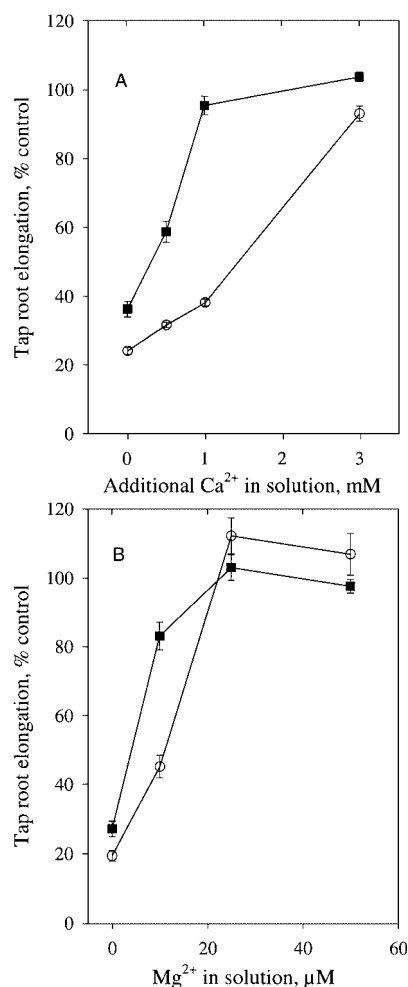
Experiments were performed to characterize tap root elon-

**Table 1** Total ion concentration ( $M_T$ ), GEOCHEM-predicted activities of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{La}^{3+}$  and  $\text{Al}^{3+}$  in solution ( $\{M\}_e$ ) and root membrane potential ( $E_0$ ) and ionic activity ( $\{M\}_m$ ) estimated by the Gouy-Chapman-Stern model as influenced by solution treatments

$\text{Ca}_T$	$\{\text{Ca}\}_e$	$\text{Mg}_T$	$\{\text{Mg}\}_e$	$\text{Mn}_T$	$\{\text{Mn}\}_e$	$\text{Al}_T$	$\{\text{Al}^{3+}\}_e$	$\text{La}_T$	$\{\text{La}^{3+}\}_e$	$\{\text{Ca}^{2+}\}_m$	$\{\text{Mg}^{2+}\}_m$ or $\{\text{Mn}^{2+}\}_m$	$\{\text{Al}^{3+}\}_m$ or $\{\text{La}^{3+}\}_m$	$E_0$ (mV)
$\mu\text{M}$													
800	578					0	0			3,213		0	-22
800	578					2.3	0.6			2,085		4.0	-16
800	578					5.8	1.5			1,519		6.2	-12
800	578					11.6	2.9			1,126		7.9	-8.6
800	578					18.7	4.7			894		9.0	-5.6
800	578					11.6	2.9			1,126		7.9	-8.6
1,300	912					11.7	2.9			1,623		6.9	-7.5
1,800	1,230					11.8	2.9			2,032		6.1	-6.6
3,800	2,386					12.5	2.9			3,187		4.4	-4.0
800	578	0	0			11.6	2.9			1,126	0	7.9	-8.6
800	578	10	7.3			11.7	2.9			1,120	14.2	7.9	-8.5
800	577	25	18.3			11.7	2.9			1,117	35.5	7.8	-8.5
800	576	50	36.6			11.7	2.9			1,104	70.2	7.8	-8.4
800	576	50	36.6			0	0			3,121	198.5	0	-21.7
800	576	50	36.6			2.4	0.6			2,030	129.1	3.9	-16.2
800	576	50	36.6			5.9	1.5			1,488	94.6	6.1	-12.2
800	576	50	36.6			18.7	4.7			879	55.9	8.9	-5.5
800	576			50	36.0	0	0			3,123	195.2	0	-21.7
800	576			50	36.0	2.4	0.6			2,030	126.9	4.0	-16.2
800	576			50	36.0	5.9	1.5			1,488	93.0	6.1	-12.2
800	576			50	36.0	18.7	4.7			879	54.9	8.9	-5.5
800	578							0	0	3,213		0	-22
800	578							1.7	0.6	2,988		6.8	-21
800	578							4.2	1.5	2,729		14.9	-20
800	578							13.7	4.7	2,121		33.5	-17
800	576	50	36.6					0	0	3,121	198.5	0	-21.7
800	576	50	36.6					1.7	0.6	2,907	184.9	6.7	-20.9
800	576	50	36.6					4.3	1.5	2,661	169.3	14.5	-19.7
800	575	50	36.6					13.7	4.7	2,107	134.0	32.7	-16.7

gation response of cv Young and the PI to a range of  $\text{Al}^{3+}$  activities in solution in the presence or absence of 50  $\mu\text{M}$  Mg. Tap root elongation was affected by solution composition and differed among soybean genotypes (Fig. 3A). In the absence of

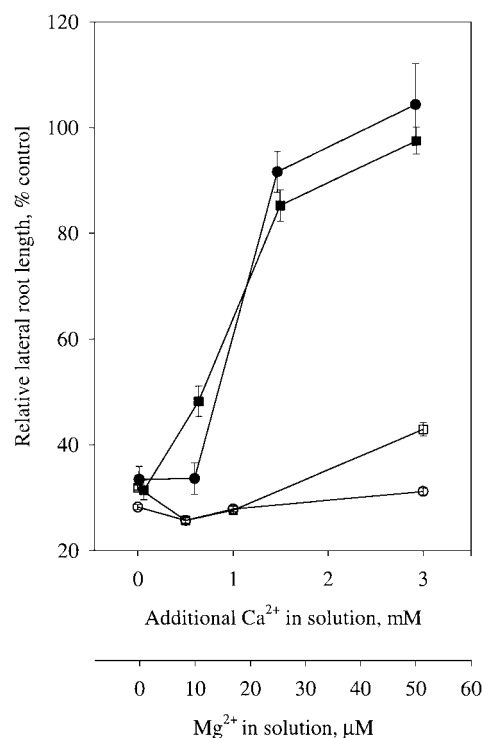
Mg, elongation of tap roots for PI was greater than for cv Young up to 1.5  $\mu\text{M}$   $\text{Al}^{3+}$  in solution. At higher  $\text{Al}^{3+}$  activities root elongation of both genotypes was reduced by over 80%. Supplementing solutions with 50  $\mu\text{M}$  Mg improved root elon-



**Fig. 1** Tap root elongation of soybean genotypes PI 416937 (filled squares) and Young (open circles) after 72 h exposure to solutions at pH 4.3 with 2.9  $\mu\text{M}$   $\text{Al}^{3+}$ , 0.8 mM  $\text{CaSO}_4$  and supplemented with variable concentrations of either Ca (A) or Mg (B). Tap root elongation (in cm/72 h) is expressed as % of control treatments without Al for each genotype and solution Ca or Mg concentration. Vertical bars denote standard errors.

gation in the low to intermediate solution  $\text{Al}^{3+}$  activities and tap root elongation of cv Young was even greater than for the PI. In solutions containing 4.7  $\mu\text{M}$   $\text{Al}^{3+}$  tap root elongation of both genotypes was greater when 50  $\mu\text{M}$  Mg was present (Fig. 3A).

Additional experiments were carried out to investigate whether the ameliorative effects of low Mg concentrations could be extended to the toxic trivalent cation La. The toxicity of La to tap roots was, in general, 15–20% less than that observed for an equivalent  $\text{Al}^{3+}$  activity in the external solution (Fig. 3B). Although the activities of  $\text{La}^{3+}$  in solution were similar to those of  $\text{Al}^{3+}$ , genotypic differences in tap root elongation were smaller than those observed with Al (compare Fig. 3A with Fig. 3B). Root elongation of the Al-tolerant PI was only slightly greater than that of cv Young when solutions had up to



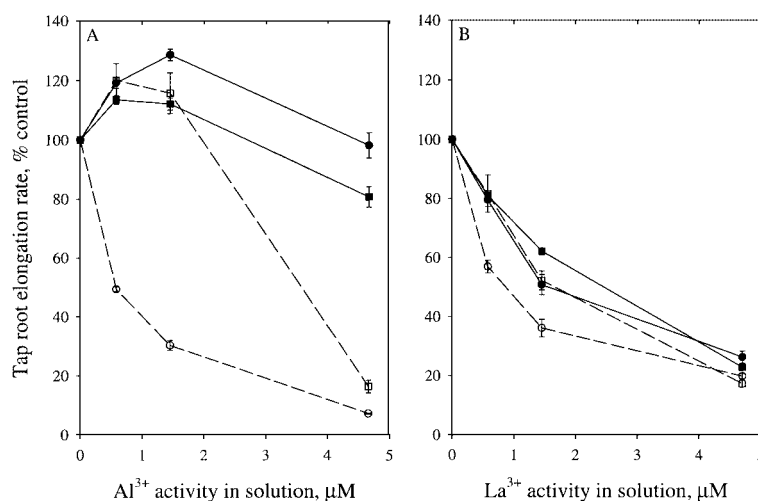
**Fig. 2** Lateral root elongation of soybean genotypes PI 416937 (open and filled squares) and Young (open and filled circles) after 72 h exposure to solutions at pH 4.3 with 2.9  $\mu\text{M}$   $\text{Al}^{3+}$ , 0.8 mM  $\text{CaSO}_4$  and supplemented with variable Ca (open symbols) and Mg (filled symbols) concentrations, relative to control treatments without Al. Vertical bars denote standard errors.

1.5  $\mu\text{M}$   $\text{La}^{3+}$ . The addition of 50  $\mu\text{M}$  Mg only had a small effect on promoting root elongation in the presence of La (Fig. 3B).

#### *Time course and specificity of Mg amelioration of Al rhizotoxicity*

Tap root elongation of cv Young and the PI were monitored over a 72 h period in solutions with a range of  $\text{Al}^{3+}$  activities with and without 50  $\mu\text{M}$  Mg (Fig. 4). When Mg was absent, tap root elongation of cv Young was consistently more sensitive to Al than the PI, except for the highest  $\text{Al}^{3+}$  activity at which tap roots of both genotypes grew poorly. Genotypic differences in Al-tolerance were detected as early as 24 h after imposing treatments (Fig. 4A, B). The addition of 50  $\mu\text{M}$  Mg to solutions allowed tap roots of cv Young to elongate as much as those of the PI across all solution  $\text{Al}^{3+}$  activities (Fig. 4C, 4D). Aluminum inhibition of root elongation was ameliorated by Mg additions as early as 24 h after starting the treatments (Fig. 4C, 4D vs. Fig. 4A, 4B).

In another set of time course experiments we investigated if Mn had a similar ameliorative effect against Al rhizotoxicity as Mg. Genotypic differences in Al tolerance among cv Young and the PI were virtually the same in both the presence and absence of 50  $\mu\text{M}$   $\text{Mn}^{2+}$  in the rooting medium. Tap root elon-



**Fig. 3** Tap root elongation relative to control treatments for soybean genotypes PI 416937 (open and filled squares) and Young (open and filled circles) after 72 h exposure to 0.8 mM  $\text{CaSO}_4$  solutions (pH 4.3) containing variable activities of  $\text{Al}^{3+}$  (A) or  $\text{La}^{3+}$  (B), with (filled symbols) or without (open symbols) 50  $\mu\text{M}$  Mg. Tap root length (cm/72 h) for control treatments with 0 and 50  $\mu\text{M}$  Mg were: Al series – 126 and 157 for PI, and 117 and 130 for Young; La series – 120 and 131 for PI, and 87 and 108 for Young. Vertical bars denote standard errors.

gation of cv Young was inhibited by Al to a greater extent than of the PI (Fig. 5A–D).

## Discussion

### *Ca and Mg amelioration of Al rhizotoxicity may involve different mechanisms*

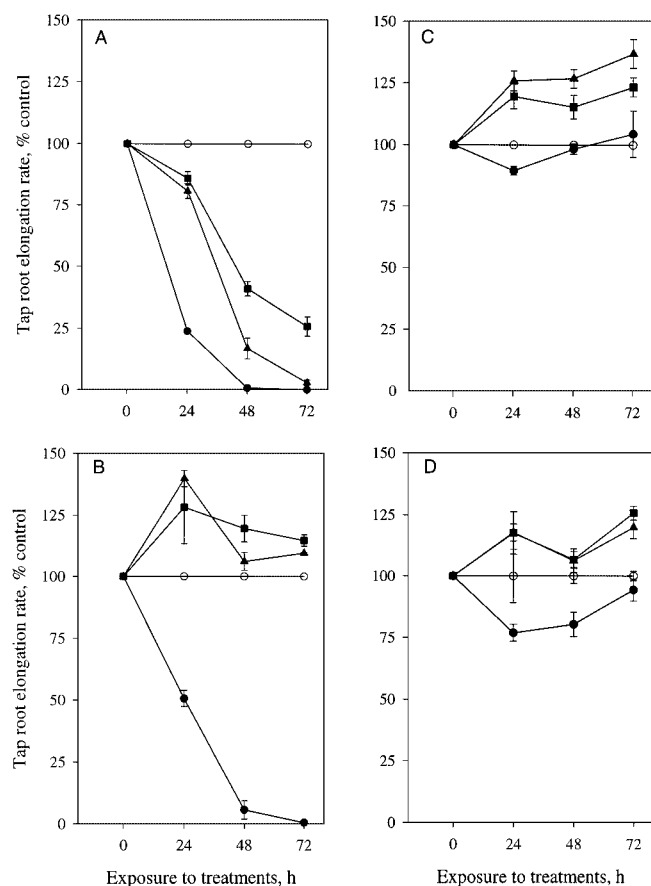
Calcium additions improved tap root elongation of both genotypes, but added Mg was approximately 100-fold more effective than Ca in alleviating Al rhizotoxicity. In the absence of Al, there was little response in root elongation to additions of Ca to the 800  $\mu\text{M}$   $\text{CaSO}_4$  background solution. Tap root elongation was improved, however, when Ca was added to solutions with 2.9  $\mu\text{M}$   $\text{Al}^{3+}$  activity (Fig. 1A). Since  $\text{Al}^{3+}$  activities in solution were kept constant across Ca treatments (Table 1), the improved root elongation of both genotypes at higher Ca levels was not due to changes in ionic strength (Edmeades et al. 1991, Noble and Sumner 1988, Wheeler and Edmeades 1995) and must have involved other beneficial effects.

Calcium amelioration of Al toxicity has been attributed to two basic mechanisms (Rengel 1992, Kinraide 1998): (1) restoration of Al-displaced Ca to non-limiting levels; and (2) increased root cell potential with a decrease of  $\text{Al}^{3+}$  at the membrane surface. In our experiments the computed electrical potential at the membrane surface became less negative, and  $\text{Al}^{3+}$  activity at the root cell surface decreased with increasing Ca levels in the growth solution (Table 1). Nevertheless, the restoration of Al-displaced Ca to non-limiting levels cannot be completely ruled out as a contributing mechanism in our studies. In prior investigation with solution Ca concentrations of 0.2–0.4 mM we attributed inhibition of tap root growth of the Al-sensitive soybean cv Essex by 9  $\mu\text{M}$   $\text{Al}^{3+}$  to an Al-induced

Ca deficiency (Silva et al. 2001b), and this effect was similar to that observed for the Al-sensitive wheat genotype Scout 66 (Kinraide 1998).

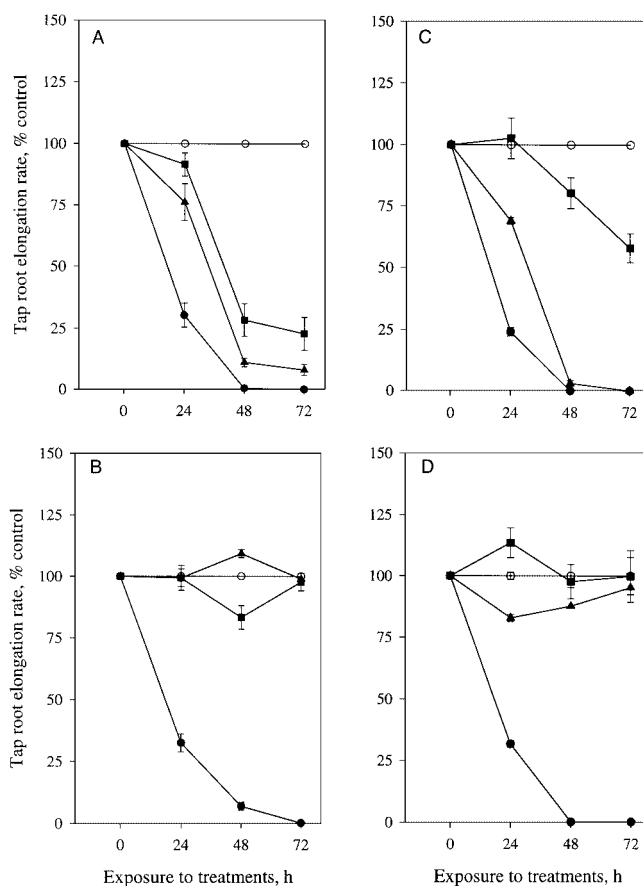
Several investigations have shown that ameliorative properties of Ca and Mg against Al toxicity for wheat roots are similar, with Ca occasionally being more effective than Mg (Kinraide et al. 1985, Kinraide and Parker 1987, Ryan et al. 1997a, Kinraide 1994, Kinraide 1998). The effects of Ca on root elongation for soybean, and the computed root cell potential and  $\{\text{Al}^{3+}\}_m$  are consistent with those reported for wheat. In contrast to Ca, however, root cell electrical potential and  $\text{Al}^{3+}$  activity computed by the Gouy-Chapman-Stern model remained virtually unchanged upon additions of Mg in the 0–50  $\mu\text{M}$  concentration range (Table 1).

In our experiments with soybean Mg may be alleviating Al damage to a critical biochemical/physiological process such as Al binding and inhibition of Mg-dependent G-proteins (Haug et al. 1994). Trivalent Al binds to the Mg binding site of heterotrimeric G-protein  $3 \times 10^7$  times more strongly than Mg and the rate of GTP hydrolysis is drastically reduced. This interferes with microtubule assembly and is thought to lead to several cellular disorders in Al-exposed cells (Macdonald and Martin 1988). In a more recent study, Landino and Macdonald (1997) demonstrated that picomolar concentrations of Al inhibited the activity of a monomeric G-protein, and increasing Mg levels in the reaction medium successfully restored the enzyme GTPase activity. Thus, it is possible that the protective effects of Mg in our study could be due to alleviation of Al binding and disruption of G protein activity that is required for normal cell activity. This would be consistent with amelioration of a symplastic lesion which has been proposed for Al toxicity (Kochian 1995).



**Fig. 4** Tap root elongation relative to control treatments for soybean genotypes Young (A and C) and PI 416937 (B and D) with time of exposure to solutions with 0 (open circles), 0.6 (filled squares), 1.5 (filled triangles) and 4.7 (filled circles)  $\mu\text{M Al}^{3+}$  (pH 4.3), in the absence (A and B) or presence (C and D) of 50  $\mu\text{M Mg}$ . Vertical bars denote standard errors.

The reasons for differences in ameliorative properties of Mg between wheat and soybean are not clear. In a previous study, we have shown that the micromolar levels of Mg alleviated Al inhibition of root elongation in soybean but not in wheat (Silva et al. 2001b). One possible explanation may reside in dissimilarities of physiological processes involved in Al-tolerance between the two species. While malate efflux by roots has been related to Al-tolerance in wheat (Delhaize and Ryan 1995), we have evidence that roots of more Al-tolerant soybean genotypes maintain higher citrate concentrations in their root tips and higher rates of citrate efflux to the culture solution (Silva et al. 1999, Yang et al. 2000). In subsequent work we show that a decrease in susceptibility to Al rhizotoxicity could result from the enhancement in citrate production and exudation by roots, and reduction in Al accumulation at the root tip of plants growing in the presence of micromolar levels of Mg in solution (Silva et al. 2001c).



**Fig. 5** Tap root elongation relative to control treatments for soybean genotypes Young (A and C) and PI 416937 (B and D) with time of exposure to solutions with 0 (open circles), 0.6 (filled squares), 1.5 (filled triangles) and 4.7 (filled circles)  $\mu\text{M Al}^{3+}$  (pH 4.3), in the absence (A and B) or presence (C and D) of 50  $\mu\text{M Mn}$ . Vertical bars denote standard errors.

#### Differential cation requirement between root classes

The greater inhibition by Al of soybean lateral root elongation as compared to the tap roots is also alleviated at higher concentrations of Ca and Mg in solution. This is in agreement with previously reported results with soybean and other species (Hecht-Bushholz and Shuster 1987, Edmeades et al. 1991, Sanzonowicz et al. 1998a, Sanzonowicz et al. 1998b, Ferrufino et al. 2000), and the differential sensitivity among root classes may be associated to a greater accumulation of Al in the tip of lateral roots than in tap roots (Lazof et al. 1994, Silva et al. 2001b).

#### Specificity of the Mg alleviation of Al rhizotoxicity

Soybean genotypes presented differential tolerance to Al, but only small differences in root elongation were observed when plants were exposed to La (Fig. 3). Addition of Mg to the growth solution elicited virtually no increase in tolerance to La

by the two genotypes (Fig. 3). Collectively, the data suggest that both genotypic tolerance and the Mg ameliorative effect are specific for Al. Since each genotype had a different tolerance to Al, but only small differences in tolerance to La, it is unlikely that their distinct sensitivity to Al is due to an intrinsic differential cell surface electrical potential (Wagatsuma and Akiba 1989). This is in agreement with previous research with Al-sensitive and Al-tolerant wheat genotypes (Delhaize et al. 1993, Kinraide et al. 1992, Kinraide 1994, Ma et al. 2000). Specificity of the Mg effect to Al may be associated with the fact that  $\text{La}^{3+}$  is not able to trigger the opening of plasma membrane anion channels, which are thought to be involved in organic acid release by roots of tolerant genotypes (Ryan et al. 1997b, Piñeros and Kochian 1999).

The ameliorative effect of Mg on protecting roots from Al injury could be detected as early as 24 h after imposing treatments. In solutions with similar  $\text{Al}^{3+}$  activities, a protective effect of Mn was virtually absent (Fig. 5). Because of similarities in coordination chemistry, Mn could potentially substitute for Mg in a series of structural and enzymatic processes in living cells. Indeed,  $\text{Mn}^{2+}$  is often used as a probe for Mg in studies investigating Mg binding sites (Martin 1990, Williams 1993, Cowan 1995). Despite their similarities, the dehydrated ionic radius of  $\text{Mn}^{2+}$  is 15% greater than that of  $\text{Mg}^{2+}$  and occupies a volume that is 53% larger than that of  $\text{Mg}^{2+}$ . Significant changes in biochemical processes may occur when Mn is substituted for Mg, since the latter forms stronger biological complexes and may not bind to the same substrates as Mg (Martin 1988, Cowan 1995). Such biochemical/physiological dissimilarities are great enough to warrant a differential effect between the two ions on alleviating Al rhizotoxicity in soybean in the present study.

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